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Pax7 reporter mouse models: a pocket guide for satellite cell research

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Abstract

Since their discovery, satellite cells have showcased their need as primary contributors to skeletal muscle maintenance and repair. Satellite cells lay dormant, but when needed, activate, differentiate, fuse to fibres and self-renew, that has bestowed satellite cells with the title of muscle stem cells. The satellite cell specific transcription factor Pax7 has enabled researchers to develop animal models against the Pax7 locus in order to isolate and characterise satellite cell-mediated events. This review focuses specifically on describing Pax7 reporter mouse models. Here we describe how each model was generated and the key findings obtained. The strengths and limitations of each model are also discussed. The aim is to provide new and current satellite cell enthusiasts with a basic understanding of the available Pax7 reporter mice and hopefully guide selection of the most appropriate Pax7 model to answer a specific research question..

Key Words: skeletal muscle; satellite cell; Pax7; mouse; stem cells.

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Human body contains more than 700 skeletal muscles, which account for up to ~40% of the total body mass in men and ~30% in females.[1] Skeletal muscle is comprised of highly specialised multinucleated myofibres formed during embryonic and foetal development.[2-4] Each myofibre contains hundreds of myofibrils which themselves consist of thousands of sarcomeres that contain the actin and myosin filaments that interact to produce force. The peripherally located myonuclei on myofibres are post-mitotic, thus as muscle precursors (myoblasts) enter terminal differentiation and fuse, the contributed nuclei lose the ability to re-enter the cell cycle. Therefore, in order to supply new myonuclei for growth, maintenance and repair, skeletal muscle relies on a population of mononuclear stem cells, adequately termed satellite cells, due to their location on the plasmalemma of a muscle fibre within the ensheathing basal lamina.[4-6] Mammalian adult skeletal muscle has a relatively slow turnover of myonuclei; hence satellite cells are mitotically quiescent. However, in response to physiological stimuli (damage, resistive exercise and disease), satellite cells are rapidly activated, providing highly proliferative myoblasts which then enter myogenic differentiation and fuse to damaged muscle fibres or form new fibres *de novo*. In order to preserve a pool of mitotically quiescent stem cells for future regenerative bouts, satellite cells undergo self-renewal.[7-10] It is impossible to mention satellite cells without mentioning the transcription factor paired-box 7 (Pax7). A member of the paired box containing family of transcription factors, Pax7 is expressed in quiescent and activated satellite cells.[11] Although other cell surface markers and their expression levels (e.g. CD34, CD29, CXCR4, c-met, M-cad) can be used to identify satellite cells, the consensus remains that the presence of Pax7 in adult mammalian muscle best identifies all satellite cells with myogenic potential.[12] Indeed, co-labelling of Pax7 with myogenic regulatory factors such as MyoD and Myogenin can help differentiate distinct aspects of the myogenic program. For example activated satellite cells express both MyoD and Pax7, whereas cells entering the differentiation phase express Myogenin but not Pax7.[9] In addition, co-immunolabelling of Pax7 with the membrane protein laminin, enables visualisation of satellite cells and the underneath myofibre they are in contact with.[13]

Although there is some debate regarding the exact role of satellite cells in adult muscle regeneration,[14-16] it is without doubt that the presence of satellite cells in regenerating muscle is indispensable.[6] Indeed, selective ablation of Pax7 positive satellite cells results in significant loss of muscle recovery post injury.[17] Furthermore, Pax7 null mice are not viable beyond 3 weeks of birth, with surviving mice being significantly smaller in size and weight.[11,18] With such an integral role,

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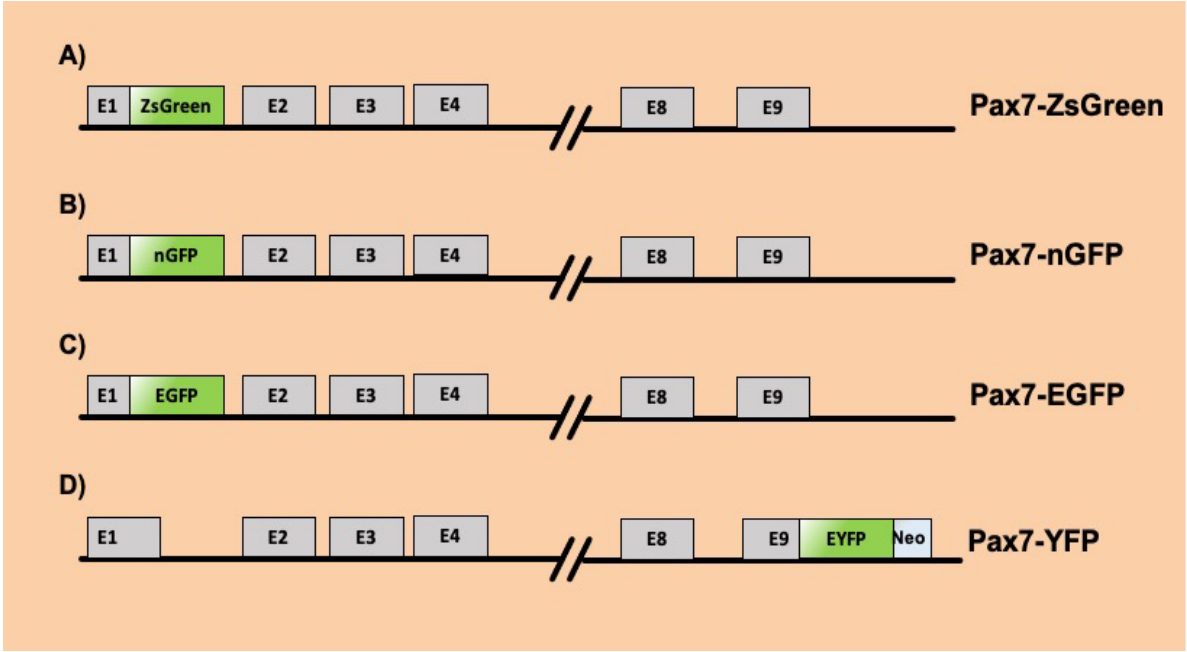

Fig 1. *Simplified schematic representing the modification of the Pax7 locus on the different reporter mice models. A) Pax7-ZsGreen, B) Pax7-nGFP, C) Pax7-EGFP and D) Pax7-YFP. E : exon Neo : Neomycin*

it is understandable that efforts to generate animal models focus on manipulation of the Pax7 gene in order to recapitulate satellite cell governed events. The mouse is the main go-to model for the development of transgenic models. In fact, mice are most frequently used to model main aspects of muscular dystrophies.[19,20] Unsurprisingly, mice comprise the majority of the current Pax7 reporter animal models. However, due to underlying complex dynamics of satellite cell regulation, choosing which Pax7 mouse model to use is key to facilitate the correct interpretation of data and understand satellite cell biology.

In this review we will introduce the currently available Pax7 reporter mice. We will highlight, using examples from key studies, how these models were developed, the main results obtained, as well as their advantages and disadvantages. The main characteristics of these models are summarised and collated in a table (Supplementary Table 1). The aim of this review is to provide a concise background and comparison of the available Pax7 reporter mice. Hopefully this will aid selection of the most appropriate model(s) to answer key questions regarding satellite cell-governed events.

Pax7-ZsGreen reporter

The heterogenous nature of satellite cells is a well-known phenomenon. Indeed, there is a distinct subpopulation of satellite cells that are more quiescent-like in nature and a subset of satellite cells more prone to enter the differentiation process.[4,21] A difference in gene expression signatures between these subgroups is believed to play a role in determining the heterogeneic functional properties of satellite cells.[4,14,22-24] To better understand the heterogenous nature of satellite cells, Bosnakovski et al, (2008) developed the Pax7-ZsGreen reporter mouse.[25] Like most of the mouse models presented in this review, bacterial artificial chromosome (BAC) modification and plasmid recombineering was used to introduce the construct of choice. Bosnakovski et al, (2008) [25] used the murine BAC library (CHORI) to obtain a BAC (RP23–218H13) containing the entire mouse Pax7 locus. This BAC was then modified (by recombineering in Escherichia coli (E. coli) to replace the first coding exon of Pax7 with a sequence encoding an enhanced green fluorescent protein, ZsGreen (Figure 1A). The modified construct was subsequently microinjected into fertilized CD1 mice oocytes. The founder transgenic Pax7-ZsGreen mice were then bred onto C57BL/6J mice. Having successfully generated the transgenic line, the first task was to assess its use as an effective reporter. Bosnakovski et al, (2008)[25] showed that as early as embryonic day 9.5 (E 9.5), the ZsGreen signal was localised to defined Pax7 expressing areas including the neural tube, frontonasal processes and the somites. From E10.5 to E12.5 the signal intensified and remained in Pax7 defined areas. In enzymatically digested muscle samples, ZsGreen positive cells could also be efficiently and robustly selected using fluorescence activated cell sorting (FACS). Indeed, FACS sorted ZsGreen positive cells congregated into a cluster of mononuclear, low granular and small sized cell population. In contrast cells from other tissues such as the

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Notexin – a neurotoxic and myotoxic phospholipase A2 derived from the venom of the Australian tiger snake, *Notechis scutatus*.

Cardiotoxin (CTX) – A cytolytic toxin isolated from the venom of the Taiwan cobra Naja, CTX is a protein kinase C inhibitor and phospholipase A2, which binds to sites on the surface of muscle cells and causes depolarization preventing muscle contraction.

Barium Chloride ($BaCl_2$) – an inorganic compound, when injected into muscles it leads to depolarization of the sarcolemma, causing Ca^{2+} overload resulting in myofiber hyper-contraction and death. $BaCl_2$ does not affect the surrounding mononuclear populations, such as the satellite cells and fibroblasts.

Text box 1. chemical agents used to induce skeletal muscle damage

heart, kidney, liver, intestine and bladder did not show any detectable fluorescence. Transcriptomic analysis showed that FACS sorted Pax7-ZsGreen positive cells had higher Pax7 expression compared to Pax7-ZsGreen negative cohorts.

A requirement of any Pax7 reporter model is to ensure that canonical satellite cell activation and myogenic progression are not hindered. With this in mind, Bosnakovski et al, (2008)[25] FACS sorted and expanded ZsGreen cells in vitro for approximately a week. Due to the reliable parameters of cell sorting of Pax7-ZsGreen positive cells, any myogenic characteristic displayed is satellite cell derived. Accordingly, Pax7-ZsGreen positive cells expanded in a typical myogenic fashion, and when differentiation was induced Pax7-ZsGreen derived cells fused forming multinucleated myotubes. Conversely, Pax7-ZsGreen negative samples adopted a fibroblastic morphology and failed to form myotubes. The nature of Pax7-ZsGreen cells was further validated by selecting and cross referencing against satellite cell associated surface markers. Indeed, Pax7-ZsGreen positive cells expressed CD29 and CD34 more abundantly than Pax7-ZsGreen negative cohorts. Additionally, three days post cardiotoxin (CTX) induced injury of the tibialis anterior (TA) muscle (text box 1), Pax7-ZsGreen sorted cells altered their surface marker expression to reflect a more myogenic activated pattern, with a lower expression of CD29 and CD34 and an increase in Sca-1 expression. A very important contribution the Pax7-ZsGreen mouse model provided to satellite cell biology, was the characterisation of a surface marker profile following in vitro expansion. Indeed, the Pax7-ZsGreen model comprehensively showed a distinct signature of Pax7-ZsGreen positive cells, consisting of higher expression of CD29 and PS-NCAM and a low expression of Sca-1 and PDGRα (i.e. $CD29^+$ $PS\text{-}NCAM^+$ $PDGR\alpha^{neg}$ $Sca\text{-}1^{neg}$). In contrast Pax7-ZsGreen negative cells displayed the opposite pattern, with high PDGRα and Sca-1 expression and relatively low expression of PS-NCAM, giving the profile: $Sca1^+$ $PDGR\alpha^+$ $PS\text{-}NCAM^{neg}$. The Pax7-ZsGreen model is also reliable for the study of the regenerative potential of satellite cells, exemplified by the successful engraftment of Pax7-ZsGreen sorted cells and the subsequent regeneration of dystrophin positive fibres in CTX injured TA muscle of dystrophin deficient mice (mdx-/- mouse model).[25]

The Pax7-ZsGreen model provides a relatively easy tool for the extraction, expansion and isolation of satellite cells. A limitation of this model is the lack of efficient ZsGreen immunofluorescent antibodies. There is also some concern over the ability of the Pax7-ZsGreen model to differentiate between satellite cell populations. For example, Bosnakovski et al (2008)[25] reported a high Myf5 and MyoD expression in Pax7-ZsGreen sorted cells. However, previous reports point to such expression patterns as indicative of a more myogenic committed subpopulation of satellite cells.[26,27] Additionally, while other reports place more emphasis on surface markers such as CXCR4 as delineating more quiescent satellite cells,[21] the Pax7-ZsGreen model does so to a lesser extent, thereby leaving some debate as to the presence of a subpopulation of satellite cells expressing higher amounts of CXCR4, which may be overlooked due to lack of resolution in the Pax7-ZsGreen mice. Recent reports also state that the Pax7-ZsGreen model is unable to clearly separate $Pax7^+$ populations at early prenatal timepoints.[28] Regardless, the expression profile presented between Pax7-ZsGreen positive and Pax7-ZsGreen negative cells, provides a useful way of identifying satellite cell derived myogenic competent

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cells. Moreover, the Pax7-ZsGreen model displays robust and reliable sorting capabilities and unaltered dynamics of satellite cell mediated events, making it a useful tool for any current or newcomer to the field of skeletal muscle research.

Pax7-n(uclear)GFP reporter

In order to get further insights into the heterogeneity of quiescent satellite cells, researchers may use the Pax7-nGFP mice.[24] To generate this model, a BAC containing the entire Pax7 locus (as well as some upstream and downstream sequences) was electroporated into the E. coli strain EL 350. Subsequent recombineering introduced a nuclear localised EGFP (nGFP), into the first exon of the Pax7 gene (Figure 1B). Fertilised eggs of Pax7-nGFP containing vectors were then microinjected into C57BL6:SJL/J mice. Offspring expressing the highest level of nGFP by epifluorescence were subsequently used for lineage maintenance and further studies. The capabilities of the Pax7-nGFP reporter are best exemplified by the work of Rocheteau et al, (2012).[21] Indeed, their initial experiments elegantly showed co-immunolabelling of GFP^+ and $Pax7^+$ cells at the satellite cell niche of adult skeletal muscle sections.[21] Through FACS sorting analysis the Pax7-nGFP model not only reliably selected all GFP positive cells but, unlike the Pax7-ZsGreen model, could further differentiate two populations at both ends of the GFP fluorescence expression spectrum. These cohorts were adequately termed Pax7-$nGFP^{Hi}$ (high GFP expression) and Pax7-$nGFP^{Lo}$ (low GFP expression). Each of these samples corresponded to approximately 10% of the total Pax7-nGFP population. With the assumption that more stem-like properties correlate with higher Pax7 expression, Rocheteau et al, (2012)[21] carried out a series of pulse-chase experiments by intraperitoneal (IP) injection of the thymidine analogue Bromodeoxyuridine (BrdU) during a five-day period. They then monitored label retention over a series of weeks (3.5 – 11.5 weeks). Remarkably, Pax7-$nGFP^{Hi}$ sorted cells retained labelling of BrdU at a higher proportion compared to Pax7-$nGFP^{Lo}$ cells, indicating that Pax7-$nGFP^{Hi}$ cohorts exhibit a slower proliferation rate. RT-qPCR analysis of FACS sorted cells revealed that Pax7-$nGFP^{Hi}$ cells expressed higher levels of Pax7 compared to Pax7-$nGFP^{Lo}$ cells. Analysis of mRNA expression also revealed that Pax7-nGFPHi cells expressed higher levels of CXCR4 and CD34 – markers associated with more quiescent stem cell populations. These findings demonstrated that the Pax7-nGFP mouse model could identify a heterogenous population of Pax7 expressing cells. Live video analysis further differentiated cellular dynamics between Pax7-$nGFP^{Hi}$ and Pax7-$nGFP^{Lo}$ cells. Indeed, a slower first division time of approximately 33 hours was noted in Pax7-$nGFP^{Hi}$ cells after their initial isolation and culture. In contrast Pax7-$nGFP^{Lo}$ cells displayed a faster first division of around 22 hours. Interestingly, subsequent divisions showed no significant differences in cell cycle time between Pax7-$nGFP^{Hi}$ and Pax7-$nGFP^{Lo}$ samples. These experiments were confirmed in vivo. CTX injured TA muscle showed that freshly isolated Pax7-$nGFP^{Lo}$ cells were almost completely MyoD positive after an overnight incubation period, whereas Pax7-$nGFP^{Hi}$ cells expressed a significantly lower (30%) proportion of MyoD. By the second day of incubation however, no discernible difference was observed between satellite cell cohorts. Rocheteau et al, (2012)[21] reasoned that a lower metabolic rate contributed to the slow activation of Pax7-$nGFP^{Hi}$ cells. Accordingly, Pax7-$nGFP^{Hi}$ cohorts displayed a lower level of mitochondrial activity as well as ATP bioluminescence compared to Pax7-$nGFP^{Lo}$ subpopulations. Taken together the Pax7-nGFP model showed that cells expressing higher amounts of Pax7 adopt a more dormant state in order to preserve stemness whereas Pax7n-$nGFP^{Lo}$ cells are primed for activation and myogenic commitment.

In a series of engraftment studies in cryo-damaged TA muscle of immunocompromised mice ($Rag2^{-/-}$:$\gamma C^{-/-}$), Rocheteau et al, (2012)[21] elegantly showed that both Pax7-$nGFP^{Hi}$ and Pax7-$nGFP^{Lo}$ subpopulations could maintain the regenerative potential up to seven rounds of serial transplantation, thus indicating an uncompromised self-renewal potential of Pax7-nGFP derived satellite cells.[21] Importantly, FACS analysis of these transplanted mice, showed that only Pax7-$nGFP^{Hi}$ cells could replenish the Pax7-$nGFP^{Hi}$ pool. The Pax7-nGFP model therefore highlighted a more stem-like population (Pax7-$nGFP^{Hi}$) upstream of more myogenic committed (Pax7-$nGFP^{Lo}$) satellite cells. The Pax7-nGFP model also enabled assessment of template DNA strand segregation (TDSS). TDSS is attributed to more stem like populations in the debated immortal DNA strand hypothesis, which suggests that; older, more “original” DNA strands from all chromosomes are favourably retained in a subset of longer lasting daughter stem cells, in an attempt of avoiding DNA damage due to multiple rounds of replication. In order to assess TDSS, Pax7-nGFP mice were first subjected to notexin (text box 1) induced injury of the TA muscle, followed by serial IP injections of 5-Ethyl-2-deoxyuridine (EdU) three days post injury for two days. BrdU was then injected to continue tracking cell division. Pax7-$nGFP^{Hi}$ and Pax7-$nGFP^{Lo}$ subpopulations were then isolated, FACS sorted and either fixed immediately after isolation (T1), or plated for 12 hours to allow cell-cycle progression and the second cell division (T2). Using this strategy, EdU incorporated DNA is considered the starting population thereby allowing comparison of DNA segregation following cell divisions. Results revealed that Pax7-$nGFP^{Hi}$ cells performed biased DNA segregation, as evidenced by the higher proportion of $BrdU^+EdU^-$ cells after two cell divisions (i.e. more Pax7-$nGFP^{Hi}$ cells retain starting DNA in a non-random manner). These findings were validated by FACS analyses which showed that after two cell divisions, Pax7-$nGFP^{Hi}$ cells had a clear non-randomised split between EdU^+BrdU^+ (56%)

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and $EdU^{-}BrdU^{+}$ (44%), whereas the Pax7-$nGFP^{Lo}$ subpopulation had an almost 100% selection for EdU^{+} $BrdU^{+}$ cells.[21] These findings suggest that Pax7-$nGFP^{Hi}$ (more stem-like) cells perform TDSS, whereas Pax7-$nGFP^{Lo}$ cells segregate their chromatids randomly. Taken together these findings suggest that cells performing TDSS are at the top of the stem cell hierarchy and can be identified by the high expression levels of Pax7 and associated stem-like markers. An elegant example of the benefit of the nGFP reporter further comes from the work by Machado et al, (2017).[29] Indeed from whole fixed muscle immediately after harvest, they were able to lysate these samples and sort for nGFP cells.[29] The major benefit was the ability to isolate satellite cells (SCs) that were in their more native state.

The Pax7-nGFP mouse model allows for efficient isolation and visualisation of Pax7 expressing cells. Importantly, the ability to differentiate reliably between subpopulations based on the nGFP reporter, provides an invaluable tool aimed at studying the heterogenous nature of satellite cells. For new researchers, FACS based experiments may be slightly challenging at first, especially when teasing apart differences within a relatively small population of cells. Therefore, allow plenty of time for training prior to carrying out extensive research. A concern when using the Pax7-nGFP model is the potential for satellite cells to alter their expression pattern in response to different stimuli such as in muscular dystrophies or other muscle damage-based protocols besides toxins or cryo-damage. Indeed, Rocheteau et al, (2012)[21] note that, they cannot rule out that under certain conditions Pax7-$nGFP^{Lo}$ satellite cells may behave like Pax7-$nGFP^{Hi}$ cohorts and perform biased DNA segregation.[21] Better understanding of how and if these Pax7 expression shifts happen will enable better handling of the Pax7-nGFP model.

The Pax7EGFP reporter

Tichy et al, (2018)[30] developed the Pax7-EGFP model with the emphasis of providing a strong Pax7 labelling marker both in vivo and in vitro – a phenotype either less pronounced or absent in the Pax7-ZsGreen, Pax7-nGFP and Pax7-YFP mouse models.[30] A BAC (RP23-204F20) containing the entire Pax7 locus (as well as DNA sequences 81kb upstream and 34kb downstream), was generated and recombineered with an enhanced green fluorescence protein (EGFP); inserted in frame immediately downstream of exon 1 of Pax7 (Figure 1C). Constructs were microinjected into fertilised eggs and implanted into recipient mice. PCR confirmed progeny were then crossed onto C57BL/6J mice. From the established lines (five in total), cells were isolated and FACS sorted. The line with the highest expression of the EGFP transgene was chosen as the founder to maintain the Pax7EGFP colony. At first glance Pax7EGFP homozygous mice ($Pax7^{EGFP/EGFP}$) may be considered a better reporter of Pax7 positive cells due to the higher levels of GFP expression compared to Pax7EGFP heterozygous counterparts ($Pax7^{EGFP/+}$). However, Tichy et al, (2018)[30] reported a significant reduction in satellite cell number in the homozygous $Pax7^{EGFP/EGFP}$ mice. Subsequent copy number variation assays (which identify the number of transgene integrations into the genome), revealed that homozygous Pax7EGFP mice have four integration sites of the EGFP transgene, compared to two in heterozygous mice. Thus, the lower satellite cell count in homozygous Pax7EGFP/EGFP mice, is likely due to enhanced instability resulting from the increased number of transgene integrations. Therefore, researchers using the Pax7EGFP reporter line should limit their experiments to heterozygous Pax7EGFP/+ mice.

The myogenic performance of the Pax7EGFP model was tested with the staple culturing of myoblasts in proliferation and differentiation conditions, followed by immunolabelling. Comparison of wild-type control (EGFP negative i.e. non-transgenic) and Pax7EGFP mice, showed no discernible difference in the proportion of cells positive for Pax7 and MyoD in proliferating myoblasts. Similarly, differentiating cells showed no change in the expression of the myogenic markers Myogenin and Myosin heavy chain (MyHC). These findings indicated that the Pax7EGFP transgene does not interfere with the myogenic process. The ability of Pax7EGFP mice to recapitulate the myogenic program was also tested in vivo. Gastrocnemius, quadriceps and TA muscles of Pax7EGFP positive and Pax7EGFP negative mice were isolated, weighed and sectioned for haematoxylin and eosin (H&E) staining. Results showed no difference in muscle weight, fibre number or cross-sectional area (CSA) between control and Pax7EGFP mice. Similarly, notexin induced injury of the TA muscle showed no significant difference in the regenerative morphologies of Pax7EGFP and wild-type control mice. Immunolabelling of these injured mice further demonstrated that over 80% of GFP positive cells could reliable co-label with the endogenous Pax7 protein. Collectively these findings showed that the Pax7EGFP model does not exhibit overt morphological differences compared to wild-type mice. Furthermore, the EGFP construct does not deviate when placed under the stress of muscle injury. The Pax7EGFP model can also be used to reliably select a satellite cell rich population through FACS. Indeed, sorting against a satellite cell marker signature consisting of $CD34^{+}$ and α7-$integrin^{+}$, Tichy et al, (2018)[30] demonstrated that over 80% of satellite cells were EGFP positive.[30] These findings are in line with previous reports using a different satellite cell marker signature in Pax7-ZsGreen mice.[31] Co-immunolabelling of FACS sorted Pax7EGFP cells, further showed clear co-expression of GFP and Pax7 under plated conditions. Pax7EGFP negative cells on the other hand showed no detectable GFP. Taken together, these findings highlight the ability of the Pax7EGFP model to selectively isolate and label satellite cells.

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A key feature of the Pax7EGFP model is the ability to track GFP expression in vitro and in vivo without the need of immunolabelling techniques. This is particularly useful as often, Pax7 immunodetection in muscle sections is a laborious task.[13,32,33] Indeed, developing Pax7EGFP embryos showed a very strong GFP signal in Pax7 localised regions. In vitro, fluorescence microscopy showed that cultured Pax7EGFP derived satellite cells expressed high levels of GFP soon after isolation, and progressively decreased in GFP expression over a four-day period – a mirroring of Pax7`s expression pattern.[30] Analysis of cryosections from adult Pax7EGFP mice continued to show a strong expression of GFP at the satellite cell niche. Additionally, two-photon microscopy of whole TA muscle samples from Pax7EGFP mice, showed an intense GFP signal in satellite cell specific regions. Importantly, GFP expression could be visualised without immunolabelling techniques. The Pax7EGFP mouse provides a reliable model for visualisation of Pax7 expressing regions. This model also enables isolation of satellite cells through FACS sorting. However, there is some concern as to how well the Pax7EGFP model can distinguish between cells that express different levels of Pax7. Indeed, the Pax7EGFP transgene provides a very strong EGFP signal which may prevent reliable isolation of heterogenous Pax7 expressing populations. In order to address this, researchers should use the Pax7-nGFP or Pax7-YFP models. New adopters of the Pax7EGFP model should remember to use heterozygous $Pax7^{EGFP/+}$ mice to avoid the aforementioned decline in satellite cells exhibited by the homozygous line.

The Pax7-YFP reporter

One of the main aims of the Pax7-YFP model was to establish a reporter line that would not alter endogenous Pax7 dynamics following insertion of the targeting vector.[34] To this end, the stop codon in exon 9 was deleted and an enhanced yellow fluorescence protein (EYFP)-loxP flanked Neo cassette (EYFP-loxP-Neo-loxP) was substituted into exon 9 of Pax7 (Figure 1D). Vectors were then injected into C57BL/6J mice, where subsequent Pax7-YFP offspring were maintained. Immunohistochemistry of $Pax7^{YFP/YFP}$ mice consistently showed clear YFP expression in Pax7 localised areas of the neural tube and dermomyotome of E10.5 embryos. However, unlike the previously mentioned Pax7 reporters (e.g. Pax7-ZsGreen), fluorescence intensity of the Pax7-YFP model was not strong enough to detect YFP in the somites of embryos. The Pax7-YFP model did however identify satellite cells in adult muscle. Indeed, fluorescence microscopy of freshly isolated soleus and extensor digitorum longus (EDL) muscle from three-month old $Pax7^{YFP/YFP}$ mice, showed positive YFP expression. Expectedly, soleus derived muscle showed greater numbers of satellite cells (YFP^+ cells) compared to the EDL, highlighting the reliability of the Pax7-YFP model. Immunolabelling of cryosections from TA muscle of $Pax7^{YFP/YFP}$ mice, further verified YFP labelling at the satellite cell niche. Moreover, quantification of co-expressing YFP^+ and $Pax7^+$ satellite cells from EDL muscle of heterozygous $Pax7^{YFP/+}$ and homozygous $Pax7^{YFP/YFP}$ derived cells, showed that all $Pax7^+$ cells were also YFP^+ and vice versa. Collectively these findings demonstrated that the Pax7-YFP model recapitulated Pax7 expression and did not hinder Pax7 protein dynamics.

It is essential for a Pax7 reporter to be able to isolate a satellite cell rich population. The Pax7-YFP model does so with great success. Indeed, Kitajima and Ono (2018)[34], showed that FACS sorted YFP positive cells adhered to a strict satellite cell marker signature (consisting of: $CD31^-$, $CD45^-$, $Sca\text{-}1^-$ and $VCAM\text{-}1^+$), thus elegantly showing that the Pax7-YFP model can competently isolate satellite cells without the need of antibody labelling. The regenerative potential of Pax7-YFP mice was also scrutinised.[34] TA muscle from adult wild-type ($Pax7^{+/+}$) and $Pax7^{YFP/+}$ mice, were subjected to CTX induced injury and analysed 3-, 7- and 14-days later. Immunolabelling showed co-expression of YFP and Pax7 as early as three days post injury. H&E staining of muscle sections at all timepoints showed no significant difference in the regenerative capacity of $Pax7^{YFP/+}$ and wild-type mice. RT-qPCR analysis also confirmed no observable change in the expression dynamics of Pax7-YFP and wild-type mice, with both cohorts displaying peak expression of Pax7, Myf5, MyoD and Myogenin three days post injury, with a subsequent decline in expression after two weeks. YFP expression itself followed this myogenic expression pattern, highlighting the ability of the Pax7-YFP model to track satellite cell activity. RT-qPCR analysis also revealed a very strong correlation between YFP and Pax7 expression – results which were confirmed at the protein level. In fact, co-immunolabelling showed almost identical fluorescence levels between Pax7 and YFP under growth medium conditions, demonstrating that the Pax7-YFP model can be used to subdivide satellite cells into high YFP (high Pax7) and low YFP (low Pax7) expressing subpopulations. Thus, similar to the Pax7-nGFP reporter, the Pax7-YFP model can assess the heterogenous nature of satellite cells.

A central objective of the Pax7-YFP model was to generate a functional homozygous line in order to minimise potential variances from altered Pax7 dynamics due to transgene insertion.[35] In order to test this, wild-type ($Pax7^{+/+}$), heterozygous ($Pax7^{YFP/+}$) and homozygous ($Pax7^{YFP/YFP}$) Pax7-YFP mice were subjected to CTX mediated damage of the TA muscle. The regenerative process was then monitored over a 14-days period. Quantification of the CSA and muscle weight, showed no significant difference in the regenerative potential of $Pax7^{YFP/YFP}$ mice. In vitro too, FACS sorted $Pax7^{YFP/YFP}$ cells showed no discernible differences in the proliferation (EdU incorporation) and differentiation (MyHC immunolabelling) phase,

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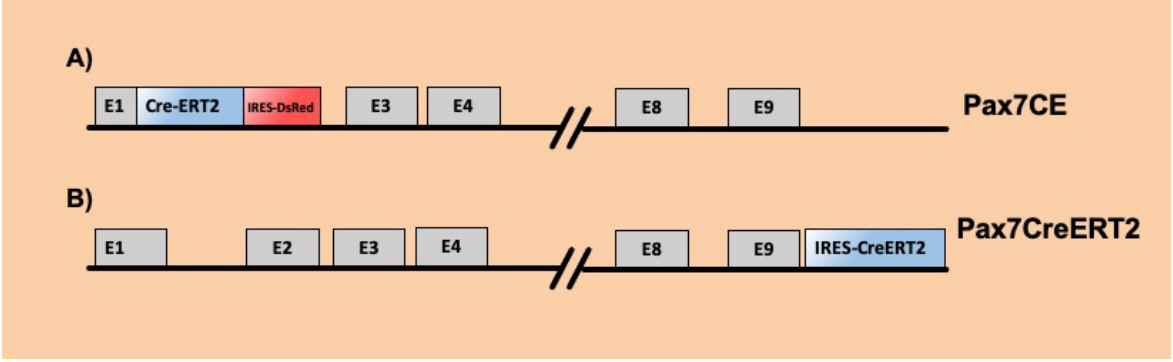

Fig 2. *Simplified schematic representing the modification of the Pax7 locus on the different inducible mice models. A) Pax7CE and B) Pax7CreERT2. E : exon IRES : Internal Ribosomal Entry Site.*

compared to wild-type ($Pax7^{+/+}$) counterparts. The self-renewal capacity of $Pax7^{YFP/YFP}$ mice during the differentiation phase also remained unchanged. Engraftment studies in mdx mice further showcased the ability of the Pax7-YFP model to study the regenerative potential of satellite cells. Indeed, mdx mice subjected to CTX induced damage of the TA muscle, showed robust regeneration of dystrophin positive myofibres, when freshly isolated FACS sorted $Pax7^{YFP/YFP}$ derived cells were transplanted into the site of injury. Additionally, YFP positive cells re-occupied their niche following the regeneration process.[34] Taken together these studies showed that $Pax7^{YFP/YFP}$ derived satellite cells employ canonical myogenic behaviour, capable of restoring injured muscle and returning to their quiescent state once their role is complete.

The Pax7-YFP model is a very reliable reporter of satellite cells. Unlike the Pax7EGFP reporter, Pax7-YFP mice do not exhibit a lower satellite cell number in the homozygous line, indicating that transgene insertion does not alter endogenous Pax7 function. This removes the burden of having to select for one genetic background over the other. However, it is advised that researchers use the homozygous $Pax7^{YFP/YFP}$ line, when trying to view EYFP expression under fluorescence microscopy without immunolabelling. Unlike the Pax7-ZsGreen reporter, the Neomycin (Neo) resistance sequence was not excised by flipase (flp) recombinase in the Pax7-YFP model. Neo resistance allows for further selection of the transgene when cells are cultured in geneticin containing medium. In summary, the Pax7-YFP reporter mouse is a good model that ticks most of the boxes for new and current scientists wishing to explore satellite cell dynamics with relative ease and precision.

Inducible Pax7 models

A common feature of all the reporter mice discussed so far is the fact that Pax7 expressing cells are marked constitutively due to the transgenes chosen. This becomes a point of concern when trying to trace the origins of different Pax7 populations during early developmental stages and beyond. Therefore, these lineage tracing analyses require a reliable system to enable the spatiotemporal reporting of Pax7. The most common way of achieving this is by generating Cre-recombinase mice.[36,37] Briefly, the Cre-recombinase gene is placed under the control of the Pax7 promoter. These mice are then crossed to mice harbouring a floxed gene – a DNA sequence inserted between two loxP sites. Subsequent Pax7 driven expression of the Cre-recombinase cleaves any gene that is floxed, thus allowing satellite cell specific regulation of a chosen transgene. This system is given an extra layer of control by making the Cre delivery inducible. The most popular being the tamoxifen (TMX) inducible Cre/loxP system. In this system, the Cre-recombinase is fused to mutated hormone-binding domains of the oestrogen receptor (ER), referred to as CreERT. Cre-mediated recombination only occurs after external administration of the drug tamoxifen which is metabolised to OHT (oestrogen receptor ligand 4-hydroxytamoxifen), thus stabilising CreERT activity. The following models employ the CreERT system to produce highly regulated Pax7 reporters.

The Pax7 CreERT2-IRES-DsRed-2XpolyA - Pax7CE model

Previously developed Pax7-Cre reporter models provide constitutive Cre activation which ultimately marks all Pax7 descendants.[38,39] Although beneficial in many respects, these models cannot be used to selectively label satellite cells at a chosen timepoint. With this in mind, Lepper and Fan (2009)[15,40] set out to develop a highly inducible Pax7 reporter mouse model. Using BAC and E. coli recombineering, a modified Pax7 locus was introduced; consisting of complete deletion of exon 2 and replacement of the coding sequence of exon 1 with a tamoxifen inducible CreERT2 sequence and an IRES-DsRed reporter (Figure 2A). Immediately downstream of the DsRed sequence there are two polyA signals (2XpolyA) – providing further stabilisation of the resulting mRNA product. The CreERT2-IRES-DsRed-

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2XpolyA progeny (hereafter referred to as Pax7^{CE}) were maintained in a C57BL/6J background. The DsRed reporter was unable to be detected either live or by antibody immunolabelling, thus the Pax7^{CE} model relies solely on crossing with other reporter mice for visualisation of Pax7 descendants. In their analysis, Lepper and Fan (2010) crossed offspring of the Pax7CE model to the Rosa26-LacZ (R26R $^{LacZ /+}$) reporter line. These mice have a LoxP pair flanking a neomycin sequence upstream of a LacZ coding sequence. Upon tamoxifen induction, Cre-mediated recombination excises the LoxP sites and enables LacZ expression only in Pax7 expressing cells. Thus, using X-gal histochemical assays, the activity of β-galactosidase (β-gal) can be visualised in Pax7 expressing descendants (40). Pregnant Pax7^{CE}/+; R26R $^{LacZ /+}$ mice were given a single tamoxifen dose one day prior the desired visualisation of embryos. Whole mount X-gal staining of very young (E6.5) embryos showed no detectable β-gal^{+} cells. From E9.5 onwards however, a clear recapitulation of Pax7 localised regions were observed. Indeed, at E9.5, β-gal^{+} cells were located in the craniofacial region, dorsal brain, neural crest cells and anterior somites. Analysis of embryos from E11.5, E12.5, E13.5 and E14.5 showed continued expression of β-gal^{+} cells at the craniofacial region and elegantly showed a shift in β-gal staining in an anterior to posterior direction at the dorsal spinal cord. X-gal staining at E15.5 and E16.5 clearly detected β-gal^{+} cells at the hindlimbs. These findings showed that the Pax7CE model could faithfully recapitulate endogenous Pax7 expression in the embryo. Importantly only tamoxifen induced Pax7$^{CE/+}$ mice could visualise β-gal activity, indicating a tightly regulated Cre recombinase in the Pax7CE model. Although informative, these initial one-day lineage experiments are quite static in their expression of Pax7 and do not necessarily establish a direct lineage relationship between early Pax7^{+} and later Pax7^{+} cells. In order to address this, Lepper and Fan (2010) carried out intermediate-term lineage tracing experiments; consisting of tamoxifen injection at various timepoints starting at E9.5 and monitoring β-gal expression at a set time point (E16.5). Intriguingly, results showed a clear shift in commitment of Pax7 expression from more widespread areas such as brown adipogenic tissue (BAT), to more muscle specific areas from E12.5 onwards, demonstrating that Pax7 descendants have a multitude of developmental potentials early on, but later become restricted to muscle specific regions.[40] These findings are supported by previous studies which showed that early muscle precursors are multipotent in nature.[41] Immunolabelling of β-gal and MyHC in lineage traced embryos at E9.5 and E11.5, showed that only E11.5 traced embryos had co-expression of β-gal and MyHC in the trunk and TA muscle of E16.5 embryos, indicating a distinct lineage of Pax7 expressing satellite cells which later contribute to trunk and limb musculature.[41] The Pax7^{CE} model can trace the identity of these early embryonic satellite cells much later in adult life. Indeed, tamoxifen injected embryos at E11.5 traced a small population of satellite cells located at their niche in 2-month-old mice. In order to test the myogenic capabilities of these early satellite cells descendants, CTX induced damage of the TA muscle was performed in E11.5 lineage traced Pax7$^{CE/+}$; R26R$^{LacZ /+}$ mice. At 2- and 5-days post injury, pulse chase assays of EdU injection were performed and analysed 10 days post injury. Results showed that E11.5 traced β-gal^{+} cells co-labelled with Pax7 and were able to activate (incorporate EdU) efficiently following injury.[40] Furthermore, E11.5 traced satellite cells were able to re-occupy their niche following injury and co-labelled with M-cadherin (Mcad) – a cell adhesion protein located at the muscle/quiescent satellite cell junction.[42] These findings demonstrated that the Pax7^{CE} model could reliably trace a subgroup of embryonic satellite cells in adult muscle. Moreover, these cells were able to actively contribute to the myogenic program as well as self-renew, making them bonified satellite cells.

The Pax7^{CE} model provides a highly regulated expression of the transgene, allowing for spatiotemporal characterisation of a gene of interest. Although the R26R^{LacZ} mouse was used in the study presented by this review,[15,40] the Pax7^{CE} model can be crossed with other floxed mice.[43] However, anyone looking to adopt the Pax7^{CE} mouse for their analyses must be aware of the inherent limitations this model has. One such limitation is that only Pax7$^{CE/+}$ heterozygous mice are viable, with Pax7$^{CE/CE}$ homozygous mice exhibiting typical Pax7 null phenotypes.[11] Moreover the heterozygous variation of this reporter model essentially halves the expression of Pax7. Indeed, the effect of Pax7 haploinsufficiency in the Pax7CE model was elegantly highlighted by Mademtzoglou et, al 2023. In this study the authors showed a reduction in satellite cell number in Pax7$^{CE/+}$ cohorts following TMX administration which subsequently resulted in delayed regeneration post cardiotoxin (CTX) damage.[35] Importantly the PAX7CreERT2 (below) model,[17] (in which the transgene leaves Pax7 intact), did not exhibit lower SC number nor delayed regeneration in its heterozygous (Pax7CreERT2/+) variation. Lepper and Fan (2010) also described a low frequency of reporter activation by a single administration of tamoxifen in Pax7CE/$^{+}$;R26R$^{LacZ/+}$mice.[40] This issue cannot be circumvented by increasing the dosage of tamoxifen, as doing so results in a high rate of embryo lethality. Researchers should also be aware that tamoxifen may be active over a 12-hour period,[44] therefore potential changes in gene expression that occur within a smaller timeframe may be difficult to establish. Researchers looking to analyse early phases of satellite cell behaviour and skeletal muscle regeneration must carefully consider the reporter/inducible Pax7 model and plan appropriate controls settings to avoid misinterpretation of results.

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Pax7CreERT2

Taking advantage of the inducible Cre-mediated recombination system, Murphy et al, (2011), set out to develop the $Pax7^{CreERT2}$ model.[17] A BAC library (CHORI clone RP24-128A11) containing a 7.4kb fragment of the Pax7 locus was recombineered into the pStart plasmid. An IRES-CreERT2-FRT-Neo-FRT cassette was then introduced 8 base pairs (bp) after the endogenous stop codon (Figure 2B). Thus, insertion of the transgene did not interfere with endogenous Pax7 expression and utilised the 3` UTR and polyA signal for mRNA stability. Targeting vectors were electroporated onto embryonic stem cells (G4 ES cells) and confirmed by southern blotting. A positive clone was then selected to establish the $Pax7^{CreERT2}$ line in a C57BL/6J background. The Neo cassette was removed by crossing $Pax7^{CreERT2+neo}$ mice with $R26R^{Flpe}$ mice, generating the $Pax7^{CreERT2}$ model.[17] In order to test the efficiency of Cre-mediated recombination in Pax7 expressing cells, Murphy et al, (2011)[17], crossed the $Pax7^{CreERT2}$ line with various reporter mice ($Pax7^{CreERT2/+}$; $R26R^{reporter/+}$) including; $R26R^{YFP}$, $R26R^{mTmG}$, $Polr2a^{nlacZ}$ and $R26R^{lacZ}$, which express cytoplasmic YFP, membrane bound GFP and β-galactosidase upon Cre activation.[45-48] Isolation of the TA muscle from tamoxifen induced $Pax7^{CreERT2/+}$; $R26R^{YFP/+}$ mice, showed robust (95%) co-immunolabelling of YFP and Pax7. These findings were confirmed by co-immunolabelling of YFP with the satellite cell markers Syndecan-4 (Syn4) and CD34. Similarly, tamoxifen administration in $Pax7^{CreERT2/+}$; $R26R^{mTmG/+}$ mice showed clear co-immunolabelling of GFP and Pax7 in cryosections of 2-month-old mice. These GFP positive cells were located at the satellite cell niche, as indicated by laminin immunolabelling. Notably, Murphy et al, (2011)[17] showed that in the absence of tamoxifen, no unwanted Cre-mediated recombination took place. Akin to the Pax7-YFP reporter, the $Pax7^{CreERT2}$ model can make use of homozygous transgenic mice ($Pax7^{CreERT2/CreERT2}$) without affecting the myogenic program. Indeed, $Pax7^{CreERT2/CreERT2}$; $R26R^{YFP/+}$ mice showed successful co-immunolabelling of YFP and CD34. Importantly, there was no difference in the total number of satellite cells between $Pax7^{CreERT2/CreERT2}$; $R26R^{YFP/+}$ and $Pax7^{CreERT2/+}$; $R26R^{YFP/+}$ mice. Thus, unlike the Pax7EGFP and Pax7CE model, homozygous $Pax7^{CreERT2}$ mice are just as useful as their heterozygous counterparts.[35] The efficiency of the $Pax7^{CreERT2}$ model was also tested under regenerative conditions using $Pax7^{CreERT2/+}$; $Polr2a^{nlacZ/+}$ and $Pax7^{CreERT2/+}$; $R26R^{lacZ/+}$ mice. In both models, TA muscles were subjected to tamoxifen injections before and after $BaCL_2$ mediated injury (text box 1). Results showed clear β-gal$^+$ staining in regenerating fibres of the TA two weeks post injury. Taken together these findings showed that the $Pax7^{CreERT2}$ mouse is an efficient Cre-mediated model and does not cause unwanted changes to endogenous Pax7 expression dynamics.

As previously mentioned, the Cre-mediated system can target any gene of interest as long as it is floxed. Therefore, to investigate the putative interplay between satellite cells and muscle connective tissue (MCT) fibroblasts, Murphy et al, (2011)[17] crossed $Pax7^{CreERT2}$ mice onto Rosa26-DTA ($R26R^{DTA}$) mice. $R26R^{DTA}$ mice consist of a stop codon flanked by loxP sites upstream of a diphtheria toxin fragment A (DTA) encoding sequence. Thus, Pax7$^+$ cells are selectively ablated by Cre-mediated production of DTA. In order to test the efficiency of Pax7 ablation, two-month-old $Pax7^{CreERT2/+}$; $R26R^{DTA/+}$ mice were subjected to IP injections of tamoxifen before and after TA muscle damage.[17] Both $BaCl_2$ and CTX muscle damage was performed in separate mice. Muscle samples were analysed 5- and 28-days post injury and compared to injured but tamoxifen untreated $Pax7^{CreERT2/+}$; $R26R^{DTA/+}$ mice. Results showed an ablation of approximately 91% of all Pax7$^+$ cells. The significant reduction in satellite cells was undoubtedly the reason why 5 days post injury, embryonic myosin heavy chain (MyHCemb) was almost absent in $Pax7^{CreERT2/+}$; $R26R^{DTA/+}$ tamoxifen treated mice and also why even after 28 days post injury, TA muscle remained substantially smaller with few MyHC positive fibres (likely due to some satellite cells escaping Cre-mediated recombination). Pax7 ablated muscle also showed aberrant presence of MCT, a phenotype attributed to an increase in MCT fibroblasts.[17]

A point of debate in skeletal muscle injury studies is the contribution other cell populations may have on muscle regeneration. Examples of such populations include mesangioblasts and PW1$^+$/Pax7$^-$ interstitial cells (PICs), which do not express Pax7 initially but do so during certain aspects of myogenic differentiation.[14,16] In order to address this, tamoxifen induction in $Pax7^{CreERT2/+}$; $R26R^{DTA/+}$ mice was performed prior to CTX induced muscle damage.[17] Subsequent harvesting of TA muscle 28-days post injury, showed a persistent and significant lack of regeneration in tamoxifen treated mice. Importantly, regenerative morphologies were similar to $Pax7^{CreERT2/+}$; $R26R^{DTA/+}$ mice subjected to tamoxifen inductions before and after muscle damage. Therefore, these findings dispelled the likelihood of non-myogenic cells or other cell types being able to compensate significantly for the absence of Pax7$^+$ satellite cells. To further explore the interplay between satellite cells and MCT fibroblasts, Murphy et al, (2011), made use of another inducible Cre-recombinase mice, $Tcf4^{CreERT2}$. Tcf4 is a marker of MCT fibroblasts. These mice were crossed with $R26R^{DTA/+}$ mice, in order to assess the effects of fibroblast ablation following $BaCl_2$ injury of the TA muscle. Tamoxifen treated $Tcf4^{CreERT/+}$; $R26R^{DTA/+}$ mice showed that ablation of fibroblasts resulted in premature activation of satellite cells, as indicated by an initial increase in MyoD$^+$ cells three days post injury, followed by a concomitant decline in MyoD$^+$ and Pax7$^+$ cells two days later (five days post injury).

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Subsequent analysis of Tcf4 ablated muscle at 28-days post injury continued to show a slight delay in regeneration, with tamoxifen treated $Tcf4^{CreERT2/+}$; $R26R^{DTA/+}$ mice having a higher proportion of smaller muscle fibres compared to untreated controls.[17] In summary, the $Pax7^{CreERT2}$ line provides a highly regulated and Pax7 specific control of a gene of interest. The fact that the construct does not affect Pax7 gene expression adds further reassurance that the endogenous expression dynamics of Pax7 are unchanged.

Concerns of the Cre-mediated system

The $Pax7^{CreERT2}$ mouse is an incredibly useful Pax7 targeting model. However as with any Cre-mediated system, there are a few concerns.

For example, Cre-mediated recombination is not always 100% efficient. Indeed, approximately 9% of cells escaped Cre-recombinase in tamoxifen treated $Pax7^{CreERT2/+}$; $R26R^{DTA/+}$ mice. Ablation of these spared $Pax7^{+}$ cells may lead to more severe regenerative impairments.[17] Indeed, von Maltzahn et al, (2013)[49] showed that persistent Pax7 ablation through continued tamoxifen administration, resulted in a more pronounced regenerative defect. Therefore, adopters of Pax7CreERT models should carefully consider the timeframe and dosage of tamoxifen induction.

Another concern is the potential for off-target effects as Cre activity is not always restricted to the intended target cells.[37] However, Murphy et al, (2011)[17] as well as Lepper, Conway and Fan (2009)[15] showed no such unwanted "leakiness" from their $Pax7^{CreERT2}$ and $Pax7^{CE}$ models.[15,17]

There is also the issue that Cre recombination may sometimes show variable levels of efficiency between mice, even among the same litter and gender.[50] Therefore, researchers should test the recombination efficiency whenever possible before carrying out extensive experiments. Researchers should also keep in mind potential variances in gene expression when using IRES driven constructs. Indeed, in a bicistronic construct, IRES-dependent second gene expression is significantly lower than their cap-dependent (7-methyl guanosine containing) first-gene expression counterparts.[51-53] Thus, in order to maximise reporter activity, construct design should favour placement of the gene of interest in control of cap-dependent expression. The $Pax7^{CE}$ model is a clear example of such design. By placing the Cre-recombinase first, and positioning the DsRed under the control of IRES, Cre-recombination is favoured at the expense of putatively lower DsRed signal. This may partly explain why DsRed was not detected in the $Pax7^{CE}$ model.[15] In the $Pax7^{CreERT2}$ model, insertion of the Cre-recombinase as the second gene under the control of IRES, may lower the efficiency of Cre activity. However, this may be negated by the potential for two Cre transgenes in a homozygous background ($Pax7^{CreERT2/\ CreERT2}$). Any new Pax7 model designers should take care when choosing the order of gene placement in a bicistronic vector.

In conclusion, since its discovery as a marker of satellite cells, great efforts have been made to try and understand the intricate nature of the transcription factor Pax7 in satellite cell biology and skeletal muscle as a whole. Hopefully this review can assist new and current researchersreveal new and exciting features of our skeletal muscles` very own satellite cells.

List of acronyms

CD29 – Integrin subunit beta 1
CD34 – Hematopoietic Progenitor Cell Antigen CD34
CD45 – Protein Tyrosine Phosphatase Receptor Type C
C-Met – Hepatocyte growth factor receptor/tyrosine-protein kinase Met
Cre – causes recombination
CXCR4 – C-X-C chemokine receptor type 4
DsRed – Discosoma sp. Red
GFP – Green fluorescent protein
loxP – locus of X(cross)-over in P1
Mcad – M-cadherin
nGFP – nuclear localizing green fluorescent protein
PAX7 – paired box 7
PS-NCAM – Polysialylated Form of the Neural Cell Adhesion Molecule
Sca-1 – Stem cells antigen-1
Syn4 – Syndecan-4
Tcf4 – Transcription factor 4
YFP – yellow fluorescent protein
ZsGreen – Zoanthus sp. Green Fluorescent Protein
α7-integrin – alpha 7 integrin

Contributions of Authors

Y.O. Designed and conceptualised this review and carried out editing and consultation throughout. H.P.O.Q. Designed and wrote the manuscript, as well as reviewed and edited the work. S.F. Carried out consultation and editing of the manuscript.

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Conflict of Interest

The authors declare they have no financial, personal, or other conflicts of interest.t.

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Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Supplementary Table 1. *Summary features of Pax7 reporter mouse models*

Model name (strain name)	Transgenic/Pax7 gene modification	Genetic background	Reporter protein	*in vitro* visualisation	*in vivo* visualisation	FACS	Immunolabelling	Cre-mediated recombination	Viable as homologue	References
Pax7-ZsGreen (B6.Cg-Tg:Pax7-ZsGreen)1Kyba)	Insertion of a ZsGreen coding sequence into exon 1	C57BL/6J	ZsGreen	NO	YES	YES	NO	NO	NO homozygous mice display random generational silencing of the transgene	(25, 31)
Pax7^{nGFP} (Tg:Pax7-EGFP)#Taj)	Insertion of an nGFP construct into exon 1	C57BL6:SJL/J	EGFP	NO	YES	YES	YES	NO	YES but studies favour use of heterozygous mice	(21, 24)
Pax7^{EGFP} (Tg:Pax7-EGFP)	Insertion of an EGFP coding sequence into exon 1	C57BL/6J	EGFP	YES	YES very strong GFP signal is detected	YES	YES	NO	YES but transgene insertions lower satellite cell number	(30)
Pax7^{YFP} Tg:Pax7-EYFP)	Removal of stop codon at exon 9 and insertion of an EYFP-loxP flanked Neo cassette	C57BL/6J	EYFP	YES homozygous Pax7$^{YFP/YFP}$ mice show slight in vitro imaging but heterozygous Pax7$^{YFP/+}$ mice do not	YES	YES	YES	NO	YES	(34)
Pax7^{CE} (B6;129-Pax7$^{tm2.1(cre/ERT2)Fan}$/J)	Deletion of exon 2 and replacement of exon 1 for a CreERT2-IRESDsRed-2XpolyA transgene	C57BL/6J	Provided when crossed with reporter floxed mice	YES when crossed with reporter floxed mice	YES when crossed with reporter floxed mice	Only when crossed with fluorescent reporter floxed mice	Only achieved by crossing with reporter floxed mice	YES	NO	(15, 40)
Pax7^{CreERT2} (B6.Cg-Pax7$^{tm1(cre/ERT2)Gaka}$/J)	Insertion of an IRES-CreERT2 cassette 8 bp after the endogenous stop codon of exon 9	C57BL/6J	Provided when crossed with reporter floxed mice	YES when crossed with reporter floxed mice	YES when crossed with reporter floxed mice	Only when crossed with fluorescent reporter floxed mice	Only achieved by crossing with reporter floxed mice	YES	YES	(17)